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(Commemoration Issue Dedicated to  
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## Effect of a Selenium Analogue of L-Lysine on Lysine Transport of *Candida pelliculosa*

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Selenium is a homologue of sulfur and tellurium, and has both metallic and non-metallic properties. It is recognized as an essential micronutrient for mammals and some other organisms, though it is toxic at high concentrations.<sup>1)</sup> The selenium amino acids occur in nature, and most are physiologically active. Several microbial and mammalian enzymes contain a selenocysteine (2-amino-3-hydroselenopropionic acid) residue as a catalytically essential moiety.<sup>2)</sup>

Some of amino acid analogues have been reported to act as a false corepressor or a false feedback inhibitor of a key enzyme participating in biosynthesis of the corresponding amino acids, and also inhibit incorporation of the amino acids into protein.<sup>3)</sup> *Se*-( $\beta$ -Aminoethyl)-L-selenocysteine (L-selenalysine), in which the  $\gamma$ -methylene group of lysine is replaced by a selenium atom, functions as a competitive inhibitor of L-lysine in protein synthesizing systems from *Escherichia coli*, rat liver and rabbit reticulocyte<sup>4)</sup>. Recently, we found that this selenium analogue inhibits growth of *Candida pelliculosa*<sup>5)</sup> and acts as a false feedback inhibitor of homocitrate synthase (EC 4. 1. 3. 21) of the yeast<sup>5)</sup>. This paper describes effect of L-selenalysine on L-lysine transport in *C. pelliculosa* compared with L-thialysine (*S*-( $\beta$ -aminoethyl)-L-cysteine), in which a sulfur atom was substituted for the selenium atom.

L-Selenalysine·HCl was synthesized from selenocystamine·HCl and  $\beta$ -chloro-L-alanine·HCl according to the method of De Marco *et al.*<sup>6)</sup> L-Thialysine was prepared from ethylenimine and L-cysteine·HCl as described previously.<sup>7)</sup> *C. pelliculosa* (IFO 0707) was cultured in Lingens-Oltmanns' minimum medium<sup>8)</sup> at 28°C with reciprocal shaking. The cells harvested in the mid-exponential phase by centrifugation were washed three times with ice-cold 0.02 M potassium phosphate buffer (pH 6.0) containing 0.5% NaCl and 0.012% MgCl<sub>2</sub>·6H<sub>2</sub>O, and suspended in the same buffer to give 0.6 of turbidity at 660 nm (cell conc., 0.9 mg/ml, dry weight). The cell suspension was maintained in an ice bath until use, and the chilled suspension was preincubated at 30°C for 10 min prior to use. Studies on L-lysine uptake into cells were performed as follows. The uptake was initiated by the rapid addition of 0.5 ml-aliquots of the cell suspension to incubation mixture containing 20  $\mu$ moles of

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potassium phosphate buffer (pH 6.0), 1  $\mu$ mole of cycloheximide, 5  $\mu$ moles of glucose and various concentrations of [ $U$ - $^{14}$ C]-L-lysine (0.2  $\mu$ Ci) (The Radiochemical Center, Amersham) in a final volume of 0.5 ml. After incubation at 30°C for a certain period, the uptake was terminated by the addition of 0.2 ml of the cold sodium azide solution (30 mM) supplemented with 1.2 M unlabeled L-lysine to the incubation mixture. The radioactive lysine accumulated in the cells was not excreted into the incubation mixture by exogenously added unlabeled lysine when the cells were washed with the potassium phosphate buffer containing sodium azide at various time intervals up to 120 min after the termination of uptake. Then 0.6 ml-aliquot of the incubation mixture was transferred to a HA Millipore filter (25 mm in diameter, 1.2  $\mu$ m pore size) and vacuum-filtered. The cells on the filter was immediately washed with 9 ml of the buffer containing 5 mM sodium azide, and the filter was transferred to scintillation vial containing 12 ml of Aquasol. The radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer (Model 3320). The counting efficiency was 83%, and corrections for the quenching effect of samples were determined with an external standard. The rate of uptake are expressed as the amounts (nmole) of L-lysine transported per min per mg of cells, dry weight.

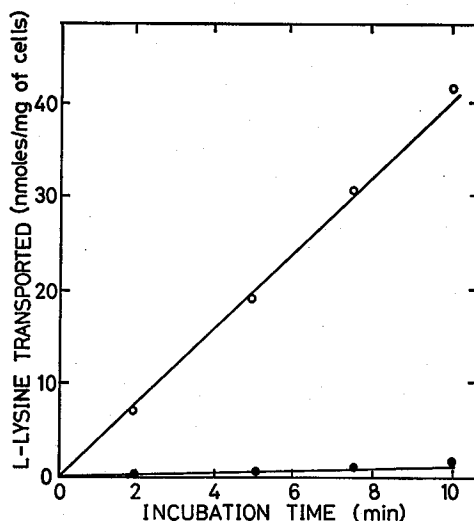


Fig. 1. Time course of L-lysine uptake by cells of *C. pelliculosa*. Preincubated cell suspensions were exposed to 200  $\mu$ M L-lysine. At the time indicated, aliquots were removed, filtered, and the total quantity of L-lysine transported was measured. Symbols: (○), None; (●), 5 mM sodium azide.

The time course of L-lysine uptake by the cells of *C. pelliculosa* is shown in Fig. 1. L-Lysine uptake proceeded linearly at least for 10 min under the conditions employed (200  $\mu$ M L-lysine was used). The addition of sodium azide (5 mM) to the incubation mixture completely inhibited the uptake. This finding suggests that the incorporation of L-lysine is mediated by an active transport. When the L-lysine concentration varied from 6.7 to 100  $\mu$ M, a saturation curve was obtained according to

the Michaelis-Menten's law. The  $K_m$  and  $V_{max}$  values were calculated to be  $13.8 \mu\text{M}$  and  $3.3 \text{ nmole/min/mg}$  (dry weight) of cells, respectively. The  $K_m$  value described above is close to that of a high-affinity lysine permease ( $K_m$ ;  $19 \mu\text{M}$ ) in *Saccharomycopsis lipolytica*<sup>93</sup>.

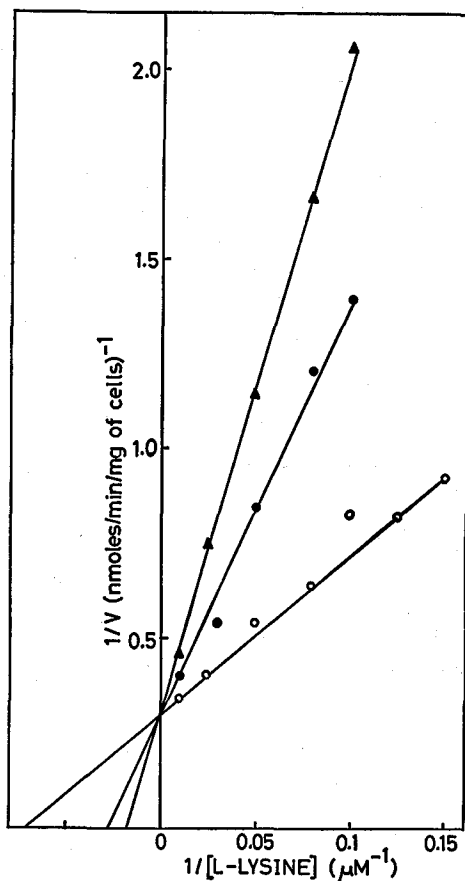


Fig. 2. Inhibition of L-lysine uptake by L-selenalysine and L-thialysine. The rate of L-lysine uptake was measured in the absence (○), or presence of  $100 \mu\text{M}$  L-selenalysine (●) or  $100 \mu\text{M}$  L-thialysine (▲).

L-Selenalysine and L-thialysine inhibited strongly the L-lysine uptake. Fig. 2 shows a double reciprocal plot of the velocity of L-lysine uptake by yeast cells *vs.* L-lysine concentration in the presence or absence of the analogues. L-Selenalysine and L-thialysine inhibited the uptake competitively with L-lysine. The inhibition constants ( $K_i$ ) for the L-lysine uptake of L-selenalysine and L-thialysine were  $59.5 \mu\text{M}$  and  $30.7 \mu\text{M}$ , respectively. L-Selenalysine is a lower competitive inhibitor against L-lysine uptake than L-thialysine in *C. pelliculosa*. We have found that L-selenalysine is a less potent competitive inhibitor for homocitrate synthase from *C. pelliculosa* compared to L-thialysine.<sup>53</sup> De Marco *et al.*<sup>43</sup> also demonstrated that L-selenalysine shows a lower inhibitory effect against L-lysine than does L-thialysine in protein

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synthesizing systems from *E. coli*, rat liver and rabbit reticulocyte. Thus, the biological effects of L-thialysine are not enhanced by substituting a selenium atom for the sulfur analogue.

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